

In the Specification:

Please replace the paragraph beginning at page 8, line 21, with the following rewritten paragraph:

b1 Figure 14A-B is a schematic representation of MEKK regulation of c-Myc controlled transcription.

Please replace the paragraph beginning at page 8, line 27, with the following rewritten paragraph:

b2 Figure 16A-B shows induction of MEKKCOOH expression by IPTG in Swiss 3T3 cells increases the number of condensed cells and stimulates c-Myc transactivation. In Figure 16A, cells were incubated in the presence or absence of 5 mM IPTG for forty eight hours. Cells were stained with acrodine orange and condensed cells quantitated per 1000 cells counted per coverslip. In Figure 16B Swiss 3T3 cells with inducible MEKKCOOH were incubated in the presence or absence of IPTG. The indicated cells were then exposed to UV-C irradiation and then fixed and stained with propidium iodide. The percentage of apoptotic cells was enumerated.

Please replace the paragraph beginning at page 9, line 1, with the following rewritten paragraph:

b3 Figure 17A-B shows that MEKKCOOH stimulates JNK/SAPK α but did not activate ERK (p42/44 MAPK) or p38Hog1. Induction of MEKKCOOH does not activate ERK (Figure 17A) or p38 (Figure 17B), whereas PDGF or sorbitol, (used as positive controls) do. Activation of the cells with PDGF or sorbitol activated ERK and p38/Hog1 as a control.

Please replace the paragraph beginning at page 9, line 5, with the following rewritten paragraph:

B4
Figure 18A-B shows that induction of MEKKCOOH expression did not significantly increase Gal4/Jun transactivation (Figure 18A). Transient transfection of MEKKCOOH resulted in increased Gal4/Jun transactivation in the MEKK2 Swiss 3T3 clone Figure 18B.

Please replace the paragraph beginning at page 9, line 8, with the following rewritten paragraph:

B5
Figure 19A-B shows that competitive inhibitory JNK/SAPK(APF) attenuates Gal4/Jun but not Gal4/myc activation. The results are representative of three independent experiments where a three-fold excess of JNK/SAPK(APF) inhibited approximately 65% of Gal4/Jun activation (Figure 19A) with no effect on Gal4/myc activation (Figure 19B).

Please replace the paragraph beginning at page 9, line 20, with the following rewritten paragraph:

B6
Figure 24A-B shows that TNF induces apoptosis in L929 cells and that this effect is blocked by bFGF. In Figure 24A cells were treated with the indicated concentrations of TNF α for 15 hours and were assayed for uptake of neutral red. In Figure 24B cells were untreated (solid bars), treated with 0.5 ng/ml bFGF (dotted bars) or 5.0 ng/ml bFGF (hatched bars) and the indicated concentrations of TNF α for 18 hours. Cell viability was assessed by neutral red assay.

Please replace the paragraph beginning at page 9, line 26, with the following rewritten paragraph:

B7
Figure 25A-B shows the activation of MAPK in L929 cells. In Figure 25A the time course of MAPK activation is shown. MAPK was isolated from cell lysates on DEAE sephacel columns and MAPK activation was measured by phosphorylation of the EGFR peptide substrate. Figure 25B depicts the concentration curve of MAPK activation by TNF α . Cells were treated with the indicated concentration of TNF α and MAPK was assayed.

Please replace the paragraph beginning at page 9, line 35, with the following rewritten paragraph:

B8
Figure 27A-B shows that bFGF does not inhibit TNF α stimulation of JNK activity. In Figure 27A serum starved L929 cells were treated as indicated. Radiolabel incorporated into GST-Jun is expressed in arbitrary phosphorimaging units. In Figure 25B cells were stimulated as indicated and assayed for MAPK activity.

Please replace the paragraph beginning at page 10, line 3, with the following rewritten paragraph:

B9
Figure 28A-B shows the effect of dominant negative N17 Ras or constitutively active V12 Ras on MAPK and JNK activities. In Figure 28A cells were uninduced (-) or induced (+) to express N17 Ras by overnight treatment with 5 mM IPTG. The cells were unstimulated(-) or stimulated(+) for 10 min with 0.5ng/ml bFGF. MAPK activity was assayed. In Figure 28B 41.LAC1 or V12 Ras cells were induced with IPTG, stimulated as indicated and analyzed for MAPK activation.

Please replace the paragraph beginning at page 10, line 15, with the following rewritten paragraph:

B10
Figure 30A-B shows the inhibition of MAPK activity and elimination of the bFGF protective effect of treatment with the MEK-1 inhibitor PD #098059. In Figure 30A serum starved L929 cells were untreated or treated for 1 hour at 37°C with the MEK-1 inhibitor (PD) and then unstimulated or stimulated with bFGF. MAPK activity was measured. In Figure 30B L929 cells were untreated or treated for 1 hour at 37°C with PD and then were untreated or treated with TNF α alone or in combination with bFGF for 18 hours. Cell viability was assessed by neutral red assay.

Please replace the paragraph beginning at page 95, line 5, with the following rewritten paragraph:

B11
The transfected cells were incubated overnight and then lysed using methods standard in the art. The luciferase activity of each cell lysate was measure on a luminometer. The results shown in Figure 13 indicate that MEKK is selectively capable of stimulating the phosphorylation of c-Myc transactivation domain in such a manner that the c-Myc domain is activated and induces transcription of the transfected luciferase gene. In addition, the results indicate that MEKK does not stimulate CREB activation. Also, activated Raf is unable to stimulate Myc activation. A schematic representation of the activation mechanism of c-Myc protein by MEKK is shown in Figure 14A-B.

Please replace the paragraph beginning at page 102, line 25, with the following rewritten paragraph:

B12
It was found that IPTG-induced MEKK_{COOH} expression stimulated signal transduction pathways that made the cells significantly more sensitive to stresses that induce cell death. For example, cells expressing MEKK_{COOH} were highly sensitive to ultraviolet irradiation. Two hours after exposure to ultraviolet irradiation greater than 30% of the MEKK_{COOH} expressing cells became morphologically highly condensed and appeared apoptotic. In contrast, the population of uninduced cells showed no increase in condensed apoptotic-like cells at this time point Figure 16A-B. Thus, overnight induction of MEKK_{COOH} expression modestly increased the basal index of morphologically condensed cells and primed the cells for apoptosis in response to UV irradiation. The results indicate that MEKK-regulated signal transduction pathways enhance apoptotic responses to external stimuli.

Please replace the paragraph beginning at page 103, line 1, with the following rewritten paragraph:

B13
C. Expression of MEKK_{COOH} stimulates JNK/SAPK and the transactivation of c-Myc and Elk-1. The ability of MEKK_{COOH} but not BxBRaf expression to induce cell death indicates that each kinase regulates different sequential protein kinase pathways. Cells were incubated for 17 hours in the absence or presence of IPTG and assayed for JNK/SAPK activity. The induction of MEKK_{COOH} expression in Swiss 3T3 cells, as predicted, stimulated JNK/SAPK activity but did not activate either ERK or p38/Hog1 activity as shown in Figures 17A-B and 18A-B. The results indicate that induction of MEKK_{COOH} results in the activation of JNK/SAPK which phosphorylates GST-c-Jun. Because known substrates for JNK/SAPK are transcription factors, we assayed MEKK_{COOH} inducible clones for transactivation of specific gene transcription. Chimeric

b13
and c

transcription factors having the Gal4 DNA binding domain and the transactivation domain of c-Myc, Elk-1 or c-Jun were used for assay of MEKK_{COOH} signaling using a Gal4 promoter-luciferase reporter gene (Hibi et al. *supra*; Sadowski, I et al. (1988) *Nature* 335:563-564; Gupta et al. *supra*; Marais et al. *supra*). Surprisingly, IPTG-induced stable expression of MEKK_{COOH} markedly activated the transactivation function of c-Myc and Elk-1 but had little effect on Gal4/Jun activity as illustrated in Figure 18A-B. This result was unexpected since MEKK_{COOH} transient expression stimulated Gal4/Jun activity, indicating that transient expression of MEKK_{COOH} was capable of transactivating c-Jun function in Swiss 3T3 cells. In addition, the JNK/SAPK activity stimulated by IPTG-induction of MEKK_{COOH} correlated with the characterized JNK/SAPK enzyme by fractionation on Mono Q FPLC. Thus, MEKK_{COOH} expression in stable clones achieved with IPTG-induction selectively regulated Gal4/Myc and Gal4/Elk-1 but not Gal4/Jun even though JNK/SAPK was activated.

Please replace the paragraph beginning at page 104, line 5, with the following rewritten paragraph:

b14

To determine if JNK/SAPK activation was required for c-Myc transactivation in response to MEKK_{COOH}, Gal4/Myc activation was assayed in the presence or absence of JNK/SAPK(APF). The results are shown in Figure 19A-B. The JNK/SAPK(APF) was used as a competitive inhibitor of JNK/SAPK for activation by the immediate upstream JNK kinase/SEK-1 enzyme (Kyriakis et al. *supra*; Sluss, et al (1994). *Mol Cell. Biol.* 14:8376-8384; Lin et al (1994) *Science* 268:286-290; Sanchez et al. (1994) *Nature* 372:794-800). In transient transfection assays, expression of JNK/SAPK(APF) inhibited approximately 65% of the Gal4/Jun activation in response to MEKK_{COOH}. In contrast, expression of JNK/SAPK(APF) had no effect on MEKK_{COOH} activation of Gal4/Myc induction of luciferase activity. Thus, c-Jun transactivation appears to be independent of

B14 Cont'd
the MEKK_{COOH} stimulated pathway leading to c-Myc transactivation. Similarly, JNK/SAPK activation can be significantly inhibited with no effect on c-Myc transactivation.

Please replace the paragraph beginning at page 119, line 11, with the following rewritten paragraph:

B15
bFGF and TNF α independently regulate cytoplasmic protein kinase cascades Figure 27A-B demonstrates that 1 ng/ml TNF α has only modest stimulatory effects on MAPK activity Figure 27A. These concentrations of bFGF and TNF α give maximal activation of MAPK and JNK, respectively. Co-stimulation of L929 cells with bFGF, at concentrations that show partial protection against TNF α -mediated killing, did not alter the magnitude of JNK activation in response to TNF α . Similarly, co-stimulation of L929 cells with TNF α , at concentrations capable of causing cell death, had little or no effect on bFGF stimulation of MAPK activity Figure 27B. Thus, in relation to JNK and MAPK, TNF α and bFGF receptors independently regulate the activity of these two sequential protein kinase pathways in L929 cells.

Please replace the paragraph beginning at page 119, line 21, with the following rewritten paragraph:

B16
Inducible expression of inhibitory and activated Ras influences apoptosis Ras activation is required for many of the phenotypic responses resulting from the activation of tyrosine kinases. Signaling by the bFGF receptor involves several different effector pathways including Ras activation. To test the involvement of Ras in the bFGF protective response, the Lac Switch inducible expression system (see Methods) was used to control the expression of inhibitory N17 Ras and constitutively activated V12 Ras in L929 cells. Figure 28A-B shows the functional consequence of expressing inhibitory N17 Ras or